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EFFECTS OF COPPER ON GERMINATION AND SEEDLING GROWTH OF DIFFERENT VARIETIES OF COWPEA (*VIGNA UNGUICULATA (L.) WALP*)

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ABSTRACT

The Asian Development Bank (ADB) Present study made to assess the response of Cowpea (*Vigna unguiculata* (L.) Walp) under the influence of copper with special reference to seed germination and seedling growth. Copper was applied in the form of copper-chloride ($CdCl_2$ $5H_2O$). Various concentrations of copper (0, 5, 10, 25, 50, 100, 150 and 200 mgl^{-1}) were prepared and treated with various Cowpea varieties *viz*., CO 2, CO 6, CO 7, Paiyur-1 and VBN-2 were used for germination studies. It is evident from the results obtained that the increase in copper concentration affected all the growth parameters such as seed germination percentage, seedling length and seedling fresh weight and dry weight.

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INTRODUCTION

The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment. The released organic compounds and heavy metals are one of the key factors that exert negative influences on man and environment causing toxicity to plants and other forms of biotics and abiotics that are continually exposed to potentially toxic heavy metals (Chandra *et al*., 2010). Of the various sources of pollutants industrial effluents containing heavy metals pose a threat to the ecosystem. These metals are present in the waste water of different industries such as metal cleaning, plating baths, refineries, mining, electroplating, paper and pulp, textile and tanneries (Mistry*et al*., 2010). Heavy metals are integrated components of biosphere and thus occur naturally in soil and plants.

Five heavy metals (Fe, Zn, Cu, Mn and Mo) are essential for all higher plants. The presence of heavy metals in the environment causes deleterious effects to human beings, particularly at certain levels of exposure and absorption. Metals like copper, iron, manganese, zinc are essential for life processes whereas others like cadmium, nickel and mercury have no physiological function but often results in harmful disorders at a higher concentration (Kavitha, 2010). Our study here was confined to copper as it is probably one of the most common contaminants of soils. Moreover, copper is also one of the essential micronutrients for plant growth. It is involved in numerous physiological functions as a component of several enzymes, mainly those which participate in electron flow, catalyze redox reactions in mitochondria and chloroplasts (Hansch and Mendel, 2009). However, in excessive quantities copper becomes toxic as it interferes with photosynthetic and

respiratory processes, protein synthesis and development of plant organelles (Upadhyay and Panda, 2009). Specifically excess copper can cause chlorosis, inhibition of root growth and damage to plasma membrane permeability, leading to ion leakage (Bouazizi *et al*., 2010). This promoted our interest to explore the effects of different concentrations of copper on germination and seeding growth of Cowpea (*Vigna unguiculata* (L.) Walp).

MATERIALS AND METHODS

The certified seeds of Cowpea (*Vigna unguiculata* (L.)Walp) varieties *viz*., CO 2, CO 6, CO 7, Paiyur-1 Land VBN-2 were obtained from the Department of Pulses, School of Genetics, Tamil Nadu Agricultural University, Coimbatore, seeds with uniform size, colour and weight were chosen for the experimental purpose and stored in metal tins as suggested by Rao (1976). Twenty seeds were evenly placed in each petridishes. They were irrigated uniformly by various concentrations of the copper chloride solution in the respective petridishes. One set was irrigated with distilled water (control). All the petridishes were kept under diffused light at room temperature (28 ± 2^0C). Five replications were maintained for each concentration. The germination percentage was recovered. The emergence of radical was taken as a criterion for germination. Five seedlings from each replicate were selected for recording the morphometrical parameters such as seed germination percentage, seedling length and seedling fresh weight and dry weight. They were recorded on the 8^{th}day after germination.

RESULTS

Seed Germination Percentage

The seed Germination percentage of Cowpea variety which were recorded on 10^{th} day after sowing are represented in Table- 1. The maximum seed germination percentage of Cowpea varieties was found to be 100% in CO-7 variety at 0 mgl^{-1}. The minimum seed germination percentage of Cowpea varieties was found to be 40% in CO-6 variety at 200 mgl^{-1}. The maximum percentage of reduction over control value was found to be 54.022% in the CO-6 at 200 mgl^{-1}. The percentage of reduction over control value was found to be 1.030% in the VBN-2 variety at 10 mgl^{-1}. The F Test value calculated for the variance between varieties and among the concentrations was found to be highly significant.

Seedling length

Efficacy of copper on seedling length of Cowpea varieties are recorded in Table – 2. The highest seedling length of Cowpea was found to be 31.7 in the CO-7 variety at 0 mgl^{-1} concentration. The lowest seedling length of Cowpea was found to be 9.6 in the CO-6 variety at 200 mgl^{-1} concentration. The maximum percentage of reduction over control value was recorded 53.398% in the CO-6 variety at 200 mgl^{-1}. The minimum percentage of reduction over control value was observed 0.993% in the VBN-2 variety at 5 mgl^{-1} copper concentration. The F-test values calculated for the variance between the varieties and among the concentration were found to be highly significant.

Table 1.Seed germination percentage of varieties of cowpea (*Vigna unguiculata* (L.)Walp) seeds as affected by different concentrations of copper

Concentration in mgl¬	Name of the varieties				
	CO-2	CO-6	CO-7	PAIYUR-1	VBN-2
Control	93	87	100	89	97
5	91(-2.151)	85(-2.298)	98(-2.000)	87(-2.247)	96(-1.030)
10	90(-3.226)	83(-4.597)	96(-4.000)	86(-3.370)	94(-3.092)
25	84(-9.677)	78(-10.344)	92(-8.000)	80(-10.112)	90(-7.216)
50	77(-17.204)	70(-19.540)	89(-11.000)	72(-19.101)	87(-10.309)
100	71(-23.656)	62(-28.735)	85(-15.000)	64(-28.089)	83(-14.432)
150	63(-32.258)	51(-41.379)	78(-12.000)	53(-40.449)	71(-26.804)
200	54(-41.935)	40(-54.022)	72(-28.000)	43(-51.685)	66(-31.758)

F- Test value for the variance between the varieties 48.921**
F-test value for the variance between the treatment 85.006**
**Significant at 1 per cent level.
Percentage of reduction over control values are given in parentheses

Table 2.Total seeding length of five varieties of cowpea (*Vigna unguiculata* (L.)Walp) as affected by different concentrations of copper(cm/seedlings)

Concentration in mgl¬	Name of the varieties				
	CO-2	CO-6	CO-7	PAIYUR-1	VBN-2
Control	28.4	20.6	31.7	25.7	30.2
5	26.6(-6.338)	19.1(-7.282)	31.2(-1.577)	24.2(-5.837)	29.9(-0.993)
10	25.2(-11.268)	18.8(-8.738)	30.9(-2.524)	23.6(-8.171)	28.5(-5.629)
25	23.7(-16.549)	16.6(-19.417)	28.6(-9.779)	21.8(-15.175)	25.3(-16.225)
50	21.0(-26.056)	15.1(-26.699)	25.6(-19.243)	19.7(-23.346)	23.5(-22.185)
100	18.3(-35.563)	13.5(-34.466)	23.9(-24.606)	17.9(-30.35)	21(-30.464)
150	16.9(-40.493)	11.8(-42.718)	21.9(-30.915)	16(-37.743)	19.8(-34.437)
200	14.5(-48.944)	9.6(-53.398)	20.2(-36.909)	13.8(-46.304)	17.9(-40.728)

F1
F- Test value for the variance between the varieties 428.57**
F-test value for the variance between the treatment 294.98**
**Significant at 1 per cent level.
Percentage of reduction over control values are given in parentheses.

Table 3. Variation in seedling fresh weight (g seedlings) of five varieties of cowpea *(Vigna unguiculata* (L.)Walp) grown in different concentrations of copper

Concentration in mgl¬	Name of the varieties				
	CO-2	CO-6	CO-7	PAIYUR-1	VBN-2
Control	2.604	2.398	3.546	2.468	3.142
5	2.561(-1.651)	2.206(-8.007)	3.500(-1.297)	2.282(-7.536)	3.100(-1.337)
10	2.462(-5.453)	2.102(-12.344)	3.458(-2.482)	2.247(-8.955)	3.081(-1.941)
25	2.198(-15.591)	1.869(-22.060)	3.112(-12.239)	2.004(-18.801)	2.727(-13.208)
50	1.891(-27.381)	1.548(-35.446)	2.804(-20.925)	1.672(-32.253)	2.389(-23.966)
100	1.611(-38.134)	1.214(-49.374)	2.432(-31.416)	1.378(-44.165)	2.010(-36.028)
150	1.374(-47.235)	0.971(-59.508)	2.189(-38.268)	1.012(-58.995)	1.702(-45.831)
200	1.012(-61.136)	0.787(-67.181)	1.972(-44.328)	0.961(-61.062)	1.464(-53.405)

F1
F- Test value for the variance between the varieties 612.12**
F-test value for the variance between the treatment 700.01**
**Significant at 1 per cent level.
Percentage of reduction over control values are given in parentheses.

Table 4: Effect of different concentrations of copper on seedling dry weight (g seedlings-1) of five varieties of cowpea (*Vigna unguiculata* (L.) Walp)

Concentration in mgl¬	Name of the varieties				
	CO-2	CO-6	CO-7	PAIYUR-1	VBN-2
Control	1.165	1.132	1.221	1.149	1.190
5	1.148(-1.459)	1.120(-1.060)	1.210(-0.901)	1.136(-1.131)	1.148(-3.529)
10	1.119(-3.948)	1.034(-8.657)	1.196(-2.048)	1.110(-3.394)	1.132(-4.874)
25	1.000(-14.163)	0.748(-33.922)	1.122(-8.108)	0.826(-28.111)	1.019(-14.370)
50	0.700(-39.914)	0.588(-48.057)	0.989(-19.001)	0.665(-42.124)	0.799(-32.857)
100	0.416(-64.292)	0.322(-71.555)	0.767(-37.183)	0.372(-67.624)	0.523(-56.050)
150	0.332(-71.502)	0.268(-76.325)	0.582(-52.334)	0.303(-73.629)	0.420(-64.706)
200	0.198(-83.004)	0.121(-89.311)	0.396(-67.568)	0.142(-87.641)	0.286(-75.966)

F1
F- Test value for the variance between the varieties 186.25**
F-test value for the variance between the treatment 22.765**
** Significant at 1 per cent level
Percentage of reduction over control values are given in parentheses.

Seedling fresh weight

The impact of copper concentration on seedling fresh weight of Cowpea varieties are represented in Table – 3. The maximum weight of Cowpea variety was observed 3.54 in CO-7 variety at 0 mgl^{-1} concentration. The minimum fresh weight of Cowpea was observed in 0.78 in CO-6 variety at 200 mgl^{-1} concentration. The maximum percentage of reduction over control value was recorded 67.181% in the CO-6 variety at 200 mgl^{-1}. The minimum percentage of reduction over control value was recorded 1.337% in VBN-2 variety at 5 mgl^{-1} concentration. The F-test values calculated for the variance between varieties and among the concentration were found to be highly significant.

Dry weight

The influence of copper concentration over the dry weight of the seedling of Cowpea is represented in Table- 4. The highest dry weight of Cowpea seedling was observed 1.221 in CO-7 variety at 0 mgl^{-1}. The lowest dry weight of Cowpea seedling was observed 0.121 in CO-6 variety at 200 mgl^{-1} copper concentration. The highest percentage of reduction over control value was found 89.311% in CO-6 variety at 200 mgl^{-1} copper concentration. The lowest percentage of reduction over control value was recorded 0.901% at 5 mgl^{-1} copper concentration. The F test values calculated for the variance between the varieties and among the concentrations were found to be highly significant.

DISCUSSION

Seed germination and growth are of vital importance for continuity of plant life.

Seed germination is defined as the resumption of metabolic process. Germination of seeds and growth of nascent seedlings are dependent process accountable for initial establishment of plant (Anwar Basha and Selvaraju, 2015). Germination and early seedling development assay has been regarded as a basic experiment for evaluating the toxicity effect of any heavy metal or chemical type of plants. In the present investigation, the germination percentage in the control was found to be maximum and gradually decreased with the increase of copper chloride concentration. The reduction in germination percentage of Cowpea may be attributed to the interference of copper ions which may inhibit seed germination by exerting deleterious effect on the activities of hydrolytic enzymes involved in the mobilization of major seed reservoirs. These findings were accordance with many other studies under copper concentrations. Salvatore *et al.* (2008) also found no influence of copper on germination of *Solanum lycopersicum*, *Raphanus sativus* and *Brassica oleracea*. The same pattern of response was noticed with high level of copper concentrations have harmful effect on germination of *Raphanus sativus* (Gupta *et al*., 2001), *Zea mays* (Mahmood *et al*., 2005), *Triticum aestivum* (Singh *et al*., 2007), *Vignaradiata* (Hema and Subramani,2013), and *Medicago sativa* (Jadi and Fulekar, 2008). In addition, Souguir *et al*. (2008) and Stoinova*et al*. (2007) reported the existence of genetic variability among crop species and cultivars for copper ion stress. Reduction in seed germination can also be attributed to alterations of selection permeability proportion of cell membrane (Manivasagaperumal *et al*., 2011). The morphological parameters such as seedling length and seedling fresh weight and dry weight showed a similar trend. It showed decreasing trend with increases copper chloride concentration.

The reduction in various morphological features like shoot length and fresh and dry weight of *Vigna unguiculata* plants on account of heavy metals depends upon dosage of copper metal, texture of the soils, etc. Seedling stage is the most sensitive stage in the life of a plant and hence, more susceptible to physical and chemical adversities. Germination and seedling establishment are vulnerable stages in the plant life cycle. At this stage, seedlings are weak and tender, hence easily affected by adverse conditions. Seed germination, root and shoot elongations have been used to test the short-term phytotoxicity in crop plants (Wang and Zhou, 2005). In the present study showed a progressive decline in seedling growth, fresh weight and dry weight of seedlings with a steady increase in copper concentration. Similar decrease in shoot and root length of different plants grown in soil consist of higher level of copper have been reported (Fernandes *et al*., 1993; Saravanan *et al*., 2001; Dharam *et al*., 2007; Manivasagaperumal *et al*., 2011; Verma *et al*., 2011; Ashagre *et al*., 2013 and Chitra, 2017). The reduction in seedling length may be due to the deleterious effects of heavy metals on the hydrolytic enzymes present in the storage organs (Geetha and De-Britto, 1998). The fresh weight and dry weight of root, stem and leaves of Cowpea was also influenced by copper treatment. The decrease in biomass in excess copper treated Cowpea might be due to low protein formation, resulting in inhibition of photosynthesis, as well as hampered carbohydrate translocation (Wani *et al*., 2007). The reduction in fresh weight and dry weight may be due to copper treatment may be attributed to the decreased metabolic rate and reduce transport from the cotyledons, at the same time it may also due to the higher rate of leakage in the membrane permeability (Chitra, 2017).

Conclusion

The present investigation reveals that the various concentration of copper has drastic effects on germination and early growth. The growth of the crop plants has been highly reduced at higher concentrations. It can be concluded that the CO 7 variety of Cowpea was proved to be tolerant and CO 6 varieties was sensitive to copper treatment. Hence, the variety CO 7 can be cultivated in the soil irrigated with industrial effluent and also in contaminated area.

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